Plant Physiol. (1969) 44, 903–906

Abscission of Cotton Flower Buds and Petioles Caused by Protein From Boll Weevil Larvae

E. E. King and H. C. Lane

Crops Research Division, Agricultural Research Service,
United States Department of Agriculture, State College, Mississippi 39762

Received March 20, 1969.

Abstract. **Protein extracted from boll weevil larvae causes abscission of cotton flower buds and debladed petioles. Abscission of buds is preceded by a rapid and persistent flaring of bracts; cell proliferation is induced in the abscission zone of debladed petioles. A possible mode of transmission of the agent from larva to bud tissue is discussed.**

The boll weevil feeds and oviposits primarily in the flower buds of cotton. Oviposition inevitably leads to abscission of affected buds. Coakley *et al.* (1) recently found that the larval stage of the insect actually causes abscission, and that feeding, oviposition, or hatching of the egg *per se* induce little if any abscission. Abscission caused by the larval stage can occur when only slight damage has been done to the bud (1). Water homogenates of boll weevil larvae also induced abscission when injected into cotton flower buds (1).

We extracted a material from boll weevil larvae which is a potent agent for inducing bud and petiole abscission. This report concerns the chemical nature of the active agent, its effects on buds and debladed petioles, and how it may be transmitted from larva to bud.

Materials and Methods

Plant Materials. Cotton plants (*Gossypium hirsutum* L., cultivar Stoneville 213) were grown in the greenhouse in pots containing a mixture of peat moss, sand, and clay soil. Flower buds used in tests were from 4 to 6 mm in diameter excluding bracts; petioles were those of leaves with the youngest fully expanded blades.

Boll Weevils. Boll weevil larvae (*Anthonomus grandis* Boh.) were collected from plates of larval diet (3). These larvae were of the standard laboratory strain maintained in the mass rearing facility of the Boll Weevil Research Laboratory, State College, Mississippi.

Test Materials. Boll weevil larvae were rinsed free of medium, homogenized in a chilled mortar and lyophilized. A portion of this material was extracted at 4° with 30 ml of 0.1 M borate buffer, pH 9.2, per gram. The extract was centrifuged for 20 min at 10,000*g*, the supernate was loaded on a 5 × 60 cm column of Sephadex G-50[1] and eluted with water at 4°. Flow rate was 6 ml per min. Two fractions resulted, 1 of large molecular size (LM) which was excluded by the gel, and 1 of smaller molecules (SM) which eluted after 3.3 hr. Both fractions were then lyophilized for use as required. Lyophilized LM material was 73 % protein by weight as determined by the biuret reaction (4) using a bovine serum albumin standard. LM and SM lyophilized material was reconstituted in water, separately and in combination at a concentration of 10 mg/ml. A 25 µl application of LM at this concentration represented 0.5 larval equivalent on a dry weight basis. Reconstituted LM was brought to 90 % saturation with $(NH_4)_2SO_4$ and the precipitate was dissolved in water at a concentration of 10 mg/ml. Reconstituted LM was also heated for 5 min at 60°. Both these materials were applied to flower buds only.

To test the possibility that flower bud abscission was merely a reaction to foreign protein, we prepared 10 mg/ml aqueous solutions of the following proteins for test on buds only: bovine serum albumin, acid and alkaline phosphatases, chitinase, pronase, L-amino acid oxidase, ribonuclease and deoxyribonuclease.

To test the possibility that the abscission agent was entering flower buds by way of larval feces, large amounts of fecal material were collected from the plates of diet prior to removal of the larvae. The feces were suspended in sufficient water to permit injection by syringe, and applied to flower buds without further treatment.

Application of Materials to Flower Buds. Homogenates and solutions were injected by hypodermic syringe in 25 µl applications. The needle was inserted in a manner similar to the insertion of the ovipositor by the female boll weevil; *i.e.*, penetration was made at the widest part of the bud with the needle tip positioned in the area of the anthers. Control buds received 25 µl of water.

Injected buds were observed for a maximum of 96 hr. This limit was chosen because it has been

[1] Mention of a specific trade name is made for identification only and does not imply endorsement by the United States Department of Agriculture.

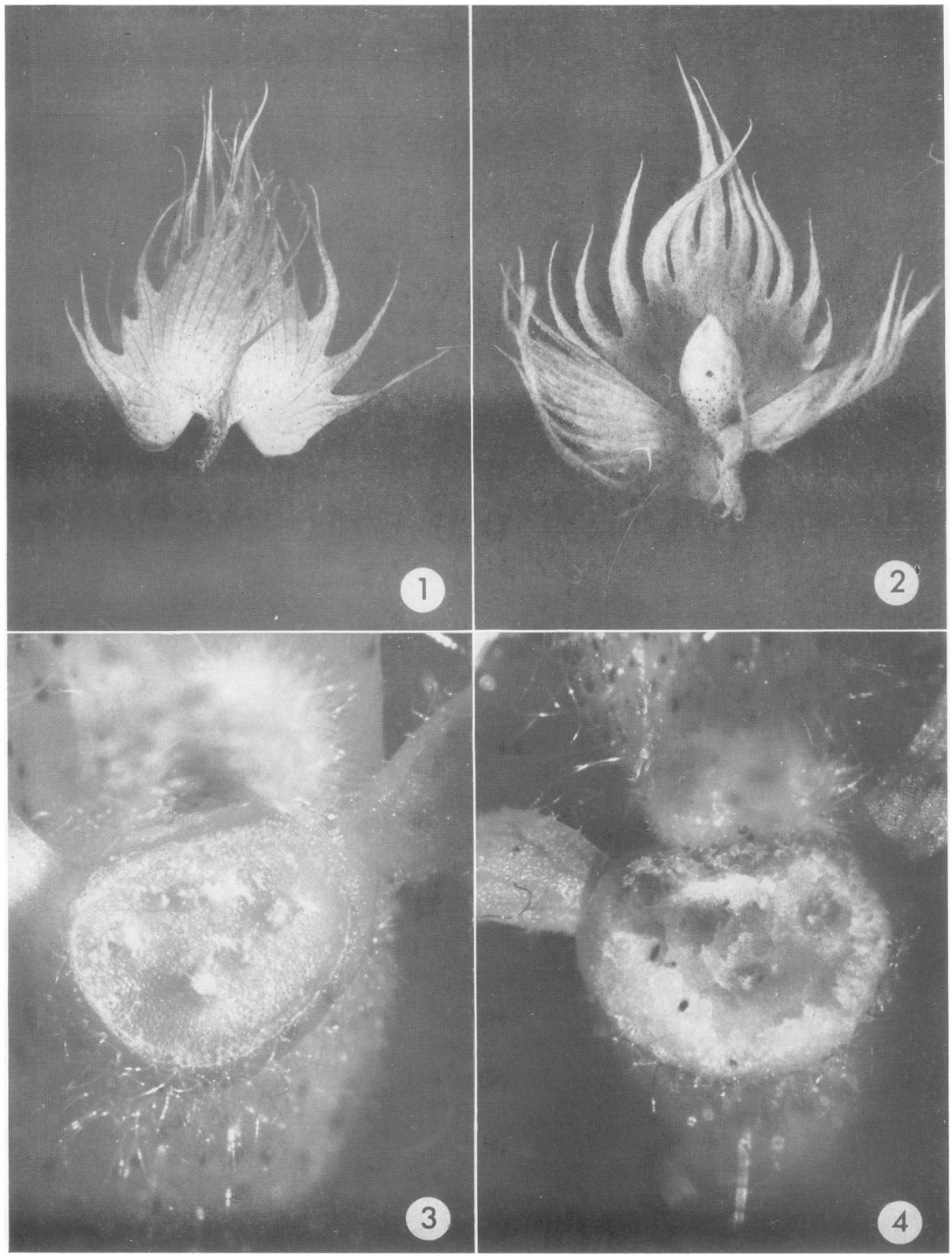

FIG. 1. Healthy flower bud, bracts not flared. ×2.
FIG. 2. Weevil-infested flower bud exhibiting flared bracts. Injection of LM material causes identical flaring. ×2.
FIG. 3. Leaf scar of control petiole. Evidence of minimal cell division present. ×20.
FIG. 4. Leaf scar of LM-treated petiole showing callus formed prior to abscission. ×20.

reported that bud abscission occurs from 84 to 118 hr after artificial implantation of boll weevil eggs under greenhouse conditions (2).

Application of Materials to Debladed Petioles. Leaf blades were removed leaving a 2 to 3 cm petiole stump. A 1.5 cm length of plastic tubing was slipped over the end of the petiole; lanolin was applied if necessary to make the connection watertight. Twenty-five μl of test material or water were introduced into the tubing. Petioles were observed for a maximum of 72 hr; after that, water controls usually began to abscise.

Results and Discussion

Chemical Nature of the Abscission Agent. Results of all tests are summarized in tables I and II. We believe that the activity of the LM fraction (73 % protein), together with the retention of activity by the $(NH_4)_2SO_4$ precipitate and its inactivation by gentle heat, are adequate criteria for establishing the nature of the abscission agent as protein. We performed a fractional $(NH_4)_2SO_4$ precipitation of this material, and obtained activity in the 40 to 60 % saturation fraction. These results, combined with the inactivity of the other proteins tested (table I) indicate that the abscission agent is protein from boll weevil larvae.

Table I. *Abscission of Flower Buds*

Buds were injected with 25 μl of test material prepared as described in the text under Materials and Methods. Results are based on 3 or 4 replications of 10 buds each test. Controls consisted of 25 μl injections of water. Control abscission was usually 0, maximum was 10 %.

Test material	Percent abscission 48 hr	96 hr
LM	50	90
SM	0	0
LM + SM	15	80
LM $(NH_4)_2SO_4$ ppt.	40	90
LM heated	0	20
Larval feces	0	10
Foreign proteins	0	10

Table II. *Abscission of Debladed Petioles*

Twenty-five μl of test material was applied as described in the text under Materials and Methods. Results are based on 3 or 4 replications of 10 petioles each test. Control petioles treated with 25 μl of water never abscissed before 72 hr, at which time tests were concluded.

Test material	Percent abscission 24 hr	72 hr
LM	70	100
SM	0	0
LM + SM	0	90

Effects of Abscission Agent on Buds and Petioles. Injection of LM material into flower buds caused a rapid and persistent flaring of the bracts prior to abscission. This flaring is identical to that exhibited by weevil-infested buds (Figs. 1 and 2).

When LM material was applied to debladed petioles, 2 effects were noted. Within 12 hr necrosis developed at the site of application, proceeding until the terminal 5 to 10 mm of the petiole stump was decomposed. The remainder of the petiole maintained a healthy appearance and retained its turgidity until abscission took place.

The responses at the base of the petiole were a pronounced swelling followed by the appearance of a break. After abscission occurred callus was evident on the separated surfaces. Figs. 3 and 4 show the greater cell proliferation on treated petioles than on controls. Seventy percent of LM-treated debladed petioles abscised within 24 hr; water-treated controls never began to abscise before 72 hr.

Surface discoloration at the point of needle puncture without damage to internal tissue is a typical response of buds injected with LM material. Abscission zones of buds show evidence of minimal cell division; callus formation occurred only in the abscission zones of petioles.

That differences in response of flower buds and petioles exist may well be due to effects of rather extensive tissue damage to petioles. We find it anomalous that the 2 types of tissue differ so greatly in their response to the LM material, yet it markedly stimulates abscission of both types of abscission zones.

Transmission of Abscission Agent From Larva to Bud. The fact that the abscission agent is a protein that is not present in fecal material (table I) or saliva (1) leads us to suggest that it is released into the flower bud tissue at the time the larva molts. We found that the most potent preparations were made from batches of second instar larvae in which some third instar forms were appearing. The presence of some third instar larvae indicates that a large number of second instar forms were nearing the molting stage, and thus the level of molting fluid in the larvae was near maximum. This fluid accumulates between the old and new cuticles, and partially digests the old cuticle prior to the molt. Molting fluid contains abundant proteins in solution, few of which have been identified (5).

Literature Cited

1. Coakley, J. M., F. G. Maxwell, and J. N. Jenkins. 1969. Influence of feeding, oviposition, and egg and larval development of the boll weevil on abscission of cotton squares. J. Econ. Entomol. 62: 244.
2. Davich, T. B., D. A. Lindquist, and J. Hacskaylo. 1965. Implanting boll weevil eggs in cotton squares for systematic insecticide and host-plant resistance studies. J. Econ. Entomol. 58: 366–68.

3. GAST, R. T. AND T. B. DAVICH. 1966. Boll weevils. In: Insect Colonization and Mass Production. C. N. Smith, ed. Academic Press, New York. p 405–18.
4. GORNALL, A. G., C. J. BARDAWEILL, AND M. M. DAVID. 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 177: 751–66.
5. WIGGLESWORTH, V. B. 1965. The Principles of Insect Physiology. 6th ed. E. P. Dutton, New York. p 41–43.